

Plasma biomarker profiles and orexin involvement in sleep phenotype and neurodegeneration in Down's syndrome

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Introduction

Alzheimer's disease (AD) pathology develops in nearly 100% of older people with Down's syndrome (DS). In recent years, evidence has begun to emerge for **sleep as a modifiable risk factor for the development of AD**. Orexin is a major regulatory neuropeptide for sleep-wake control and has been implicated in a variety of sleep disorders and neurodegenerative conditions. While CSF orexin has been shown to be significantly decreased in a small sample of DS participants compared to controls, interestingly, plasma orexin has been shown to increase with age in the general population and is significantly increased in patients with AD. Additionally, orexin-A levels positively correlated with A β 42 and pTau in the CSF of cognitively normal elderly adults. A foundational paper by Kang *et al* demonstrated not only increased interstitial fluid (ISF) A β levels during acute sleep deprivation, but also significant increases in the A β in the ISF during orexin infusion, which was normalised by dual orexin-receptor antagonism. Given this context, we sought to establish **whether plasma orexin was associated with biomarkers of AD neuropathogenesis, age, daytime sleepiness and cognition in a DS cohort**.

Methods

Plasma orexin, inflammatory mediators and AD-related biomarkers were quantified in 22 adults with DS and 18 age-matched controls. Generalised linear models using hypothesis-driven and data-driven approaches were applied to identify predictors of orexin levels. Blood samples were obtained for participants in EDTA collection tubes, and plasma was prepared by centrifuging at 20°C at 1500g for 10 minutes, 20-30 minutes after sample collection. Supernatant was aliquoted and stored immediately at -80°C. Plasma A β 40, A β 42, NfL, GFAP and pTau217 were quantified using Quanterix SIMOA (MA, USA) Neuro 4-PLEX E Advantage Plus and AlzPath pTau217 Advantage Plus immunoassays on an HDx instrument. IL-2, IL-6, IL-8, IL-10, IFN γ and TNF α concentrations were quantified using Biotechne Ella (MN, USA) Simple Plex Cytokine Screening Panel 2. Orexin was quantified by ELISA using the Abbexa (Cambridge, UK) Human Hypocretin Neuropeptide Precursor (HCRT) kit. All assays were performed according to manufacturer protocol.

Results

Compared with controls, the **DS group showed significantly increased A β 40, A β 42, pTau217, NfL and TNF α , significantly decreased A β 42:40 and significantly higher daytime sleepiness as measured by the Epworth Sleepiness Scale (ESS) ($p < 0.05$) (Fig. 1). Hypothesis-driven modelling demonstrated that **ESS, IL-8 and NfL significantly predicted orexin** in DS (Fig. 2). Data-driven modelling confirmed strong multivariate explanatory performance, with ESS being the most robust independent predictor (Fig. 3). While the two modelling methodologies yielded slightly different profiles of predictor variables and co-efficient estimates, both approaches demonstrated significant explanatory performance.**

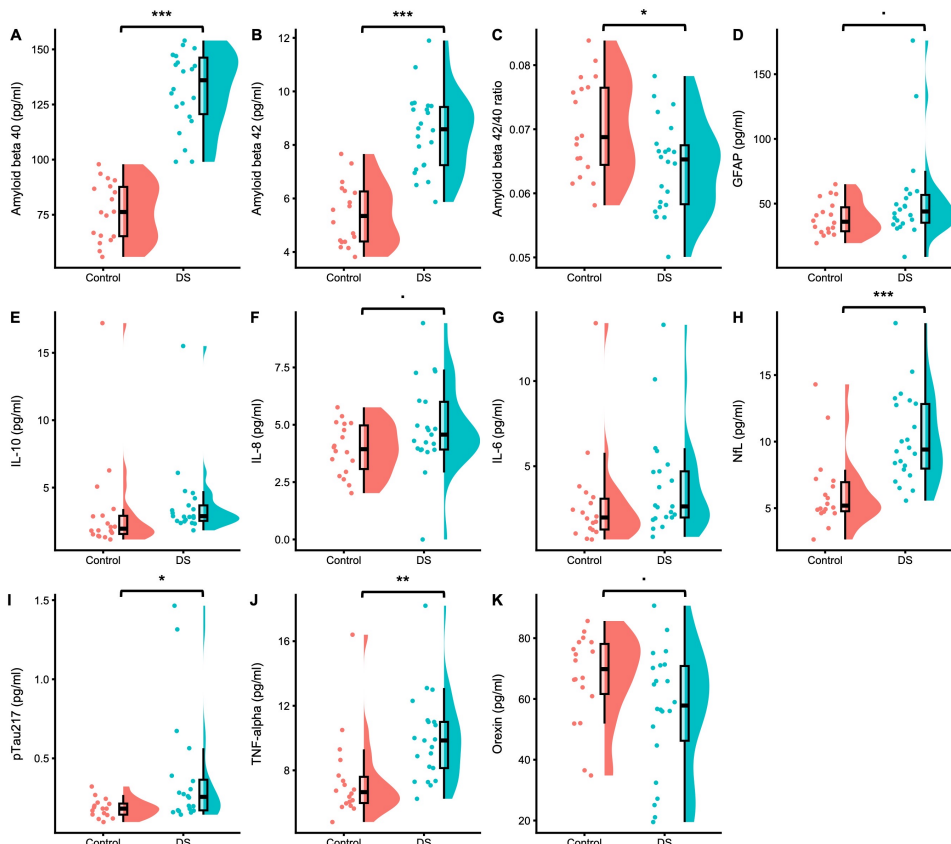


Fig. 1. Plasma biomarker quantifications by group (Control and DS): A β 40 (A), A β 42 (B), A β 42:40 (C), GFAP (D), IL-10 (E), IL-8 (F), IL-6 (G), NfL (H), pTau217 (I), TNF α (J) and orexin (K). Abbreviations: A β 40, amyloid beta 40; A β 42, amyloid beta 42; pTau217, phosphorylated tau 217; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; TNF α , tumour necrosis factor alpha. P-value significance: “.” = $p < 0.015$, “*” = $p < 0.05$, “**” = $p < 0.01$, “***” = $p < 0.001$.

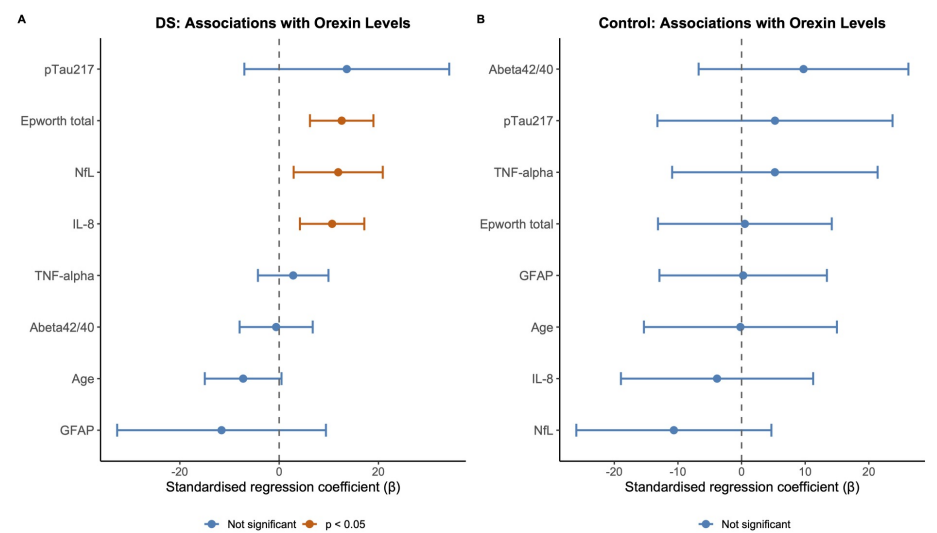


Fig. 2. Hypothesis-driven GLM regression coefficients (standardised) for DS group (A) and control group (B).

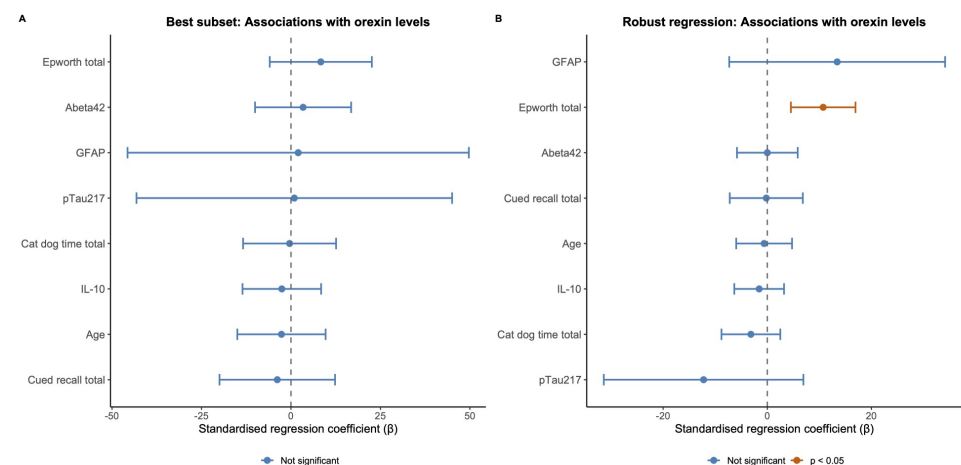


Fig. 3. Data-driven GLM regression coefficients (standardised) for best subset regression (A) and robust regression (B) in the DS group.

Discussion

We present findings that indicate significant increases in AD pathology in blood plasma in a middle-aged cohort of people with DS without MCI or AD compared to controls, demonstrating early-onset changes prior to emergence of AD symptomatology in DS, in line with known findings. Hypothesis-driven modelling of orexin prediction revealed that NfL, IL-8 and ESS scores significantly positively contributed to orexin levels, **highlighting a combination of neurodegenerative, inflammatory and sleep phenotypic explanatory variables which were not present in the low-risk control cohort**. The data-driven approach showed that within the best subset of explanatory variables no predictors were individually significant, and only the ESS score positively predicted orexin with significance when accounting for highly influential cases using a robust regression approach. This approach confirms that when optimisation of the predictors is prioritised via the modelling method, the best subset output is highly multivariate.

Convergence of findings across modelling strategies is supportive of the robustness of the identified predictors for orexin levels, whilst divergence highlights the influence of model specification on predictor selection and estimation of effects.

In conclusion, we show that orexin levels were best explained in adults with DS by a combination of sleep phenotype, cognitive performance and AD-related and inflammatory plasma biomarkers. **We suggest that orexin-signalling may be both pathologically involved in the development of AD in DS, and a targetable system for future AD treatment or prevention.**

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